### APOPTOSIS

The concept of programmed cell death, or apoptosis, has exploded into a major scientific field of interest for cell biologists, oncologists, and many other biomedical researchers. Apoptosis occurs throughout the lifetime of most multicellular organisms. During development, for example, the selective death of cells is vital to remove tissue between the digits to produce fingers and toes. Apoptosis is also necessary to destroy cells that represent a threat to the integrity of the organism, for example, cells infected by a virus. In many cancers the genes regulating apoptosis are defective, producing immortal, continuously proliferating cells. This book discusses the philosophical and technical difficulties in defining the moment of death for a cell, as well as the biological implications and significance of programmed cell death. Recent developments in the genetic control and interacting gene networks associated with apoptosis are presented. The book is written for advanced undergraduate and postgraduate students, and is highly illustrated to aid understanding.

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# Apoptosis

## THE LIFE AND DEATH OF CELLS

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In memory of Sarah

# Contents

Acknowledgements and dedicationsxv1Dead or alive1Movement1Metabolism2Sensory perception3Reproduction3Cell death: human analogies72How to die15The undead15The clearly dead17Necrosis18Apoptosis20Situations where death might be initiated23How long to die?28Occurrence of apoptosis29What's in a name? A rose is a rose33Mitotic death36Apoptosis versus necrosis37DNA degradation38How do we recognise apoptosis?42Assessment of DNA fragmentation43Assessment of protease activity in apoptosis49Changes in apoptosis-regulatory proteins52	Preface		<i>page</i> xi
1Dead or alive1Movement1Metabolism2Sensory perception3Reproduction3Cell death: human analogies72How to die15The undead15The clearly dead17Necrosis18Apoptosis20Situations where death might be initiated23How long to die?28Occurrence of apoptosis29What's in a name? A rose is a rose33Mitotic death36Apoptosis versus necrosis37DNA degradation38How do we recognise apoptosis?42Assessment of DNA fragmentation43Assessment of protease activity in apoptosis49Changes in apoptosis-regulatory proteins52	Acknowledgements and dedications		XV
Movement1Metabolism2Sensory perception3Reproduction3Cell death: human analogies72How to die15The undead15The clearly dead17Necrosis18Apoptosis20Situations where death might be initiated23How long to die?28Occurrence of apoptosis29What's in a name? A rose is a rose33Mitotic death36Apoptosis versus necrosis37DNA degradation38How do we recognise apoptosis?42Assessment of DNA fragmentation43Assessment of protease activity in apoptosis49Changes in apoptosis-regulatory proteins52	1	Dead or alive	1
Metabolism2Sensory perception3Reproduction3Cell death: human analogies72How to die15The undead15The clearly dead17Necrosis18Apoptosis20Situations where death might be initiated23How long to die?28Occurrence of apoptosis29What's in a name? A rose is a rose33Mitotic death36Apoptosis versus necrosis37DNA degradation38How do we recognise apoptosis?42Assessment of DNA fragmentation43Assessment of protease activity in apoptosis49Changes in apoptosis-regulatory proteins52		Movement	1
Sensory perception3Reproduction3Cell death: human analogies72 How to die15The undead15The undead17Necrosis18Apoptosis20Situations where death might be initiated23How long to die?28Occurrence of apoptosis29What's in a name? A rose is a rose33Mitotic death36Apoptosis versus necrosis37DNA degradation38How do we recognise apoptosis?42Assessment of DNA fragmentation43Assessment of protease activity in apoptosis49Changes in apoptosis-regulatory proteins52		Metabolism	2
Reproduction3Cell death: human analogies72 How to die15The undead15The clearly dead17Necrosis18Apoptosis20Situations where death might be initiated23How long to die?28Occurrence of apoptosis29What's in a name? A rose is a rose33Mitotic death36Apoptosis versus necrosis37DNA degradation38How do we recognise apoptosis?42Assessment of DNA fragmentation43Assessment of protease activity in apoptosis49Changes in apoptosis-regulatory proteins52		Sensory perception	3
Cell death: human analogies72 How to die15The undead15The clearly dead17Necrosis18Apoptosis20Situations where death might be initiated23How long to die?28Occurrence of apoptosis29What's in a name? A rose is a rose33Mitotic death36Apoptosis versus necrosis37DNA degradation38How do we recognise apoptosis?42Assessment of DNA fragmentation43Assessment of protease activity in apoptosis49Changes in apoptosis-regulatory proteins52		Reproduction	3
2 How to die15The undead15The clearly dead17Necrosis18Apoptosis20Situations where death might be initiated23How long to die?28Occurrence of apoptosis29What's in a name? A rose is a rose33Mitotic death36Apoptosis versus necrosis37DNA degradation38How do we recognise apoptosis?42Assessment of DNA fragmentation43Assessment of protease activity in apoptosis49Changes in apoptosis-regulatory proteins52		Cell death: human analogies	7
The undead15The clearly dead17Necrosis18Apoptosis20Situations where death might be initiated23How long to die?28Occurrence of apoptosis29What's in a name? A rose is a rose33Mitotic death36Apoptosis versus necrosis37DNA degradation38How do we recognise apoptosis?42Assessment of DNA fragmentation43Assessment of protease activity in apoptosis49Changes in apoptosis-regulatory proteins52	2	How to die	15
The clearly dead17Necrosis18Apoptosis20Situations where death might be initiated23How long to die?28Occurrence of apoptosis29What's in a name? A rose is a rose33Mitotic death36Apoptosis versus necrosis37DNA degradation38How do we recognise apoptosis?42Assessment of DNA fragmentation43Assessment of protease activity in apoptosis49Changes in apoptosis-regulatory proteins52		The undead	15
Necrosis18Apoptosis20Situations where death might be initiated23How long to die?28Occurrence of apoptosis29What's in a name? A rose is a rose33Mitotic death36Apoptosis versus necrosis37DNA degradation38How do we recognise apoptosis?42Assessment of DNA fragmentation43Assessment of protease activity in apoptosis49Changes in apoptosis-regulatory proteins52		The clearly dead	17
Apoptosis20Situations where death might be initiated23How long to die?28Occurrence of apoptosis29What's in a name? A rose is a rose33Mitotic death36Apoptosis versus necrosis37DNA degradation38How do we recognise apoptosis?42Assessment of DNA fragmentation43Assessment of protease activity in apoptosis49Changes in apoptosis-regulatory proteins52		Necrosis	18
Situations where death might be initiated23How long to die?28Occurrence of apoptosis29What's in a name? A rose is a rose33Mitotic death36Apoptosis versus necrosis37DNA degradation38How do we recognise apoptosis?42Assessment of DNA fragmentation43Assessment of protease activity in apoptosis49Changes in apoptosis-regulatory proteins52		Apoptosis	20
How long to die?28Occurrence of apoptosis29What's in a name? A rose is a rose33Mitotic death36Apoptosis versus necrosis37DNA degradation38How do we recognise apoptosis?42Assessment of DNA fragmentation43Assessment of protease activity in apoptosis49Changes in apoptosis-regulatory proteins52		Situations where death might be initiated	23
Occurrence of apoptosis29What's in a name? A rose is a rose33Mitotic death36Apoptosis versus necrosis37DNA degradation38How do we recognise apoptosis?42Assessment of DNA fragmentation43Assessment of protease activity in apoptosis49Changes in apoptosis-regulatory proteins52		How long to die?	28
What's in a name? A rose is a rose33Mitotic death36Apoptosis versus necrosis37DNA degradation38How do we recognise apoptosis?42Assessment of DNA fragmentation43Assessment of protease activity in apoptosis49Changes in apoptosis-regulatory proteins52		Occurrence of apoptosis	29
Mitotic death36Apoptosis versus necrosis37DNA degradation38How do we recognise apoptosis?42Assessment of DNA fragmentation43Assessment of protease activity in apoptosis49Changes in apoptosis-regulatory proteins52		What's in a name? A rose is a rose	33
Apoptosis versus necrosis37DNA degradation38How do we recognise apoptosis?42Assessment of DNA fragmentation43Assessment of protease activity in apoptosis49Changes in apoptosis-regulatory proteins52		Mitotic death	36
DNA degradation38How do we recognise apoptosis?42Assessment of DNA fragmentation43Assessment of protease activity in apoptosis49Changes in apoptosis-regulatory proteins52		Apoptosis versus necrosis	37
How do we recognise apoptosis?42Assessment of DNA fragmentation43Assessment of protease activity in apoptosis49Changes in apoptosis-regulatory proteins52		DNA degradation	38
Assessment of DNA fragmentation43Assessment of protease activity in apoptosis49Changes in apoptosis-regulatory proteins52		How do we recognise apoptosis?	42
Assessment of protease activity in apoptosis49Changes in apoptosis-regulatory proteins52		Assessment of DNA fragmentation	43
Changes in apoptosis-regulatory proteins 52		Assessment of protease activity in apoptosis	49
		Changes in apoptosis-regulatory proteins	52

viii

#### CONTENTS

	Membrane changes	54
	Morphology	56
	Cell death in cell cultures	57
3	What to wear and who clears up the rubbish?	61
4	To reproduce or die?	67
	Defining our terms	67
	DNA replication	69
	Cell division	71
	How do we recognise a proliferating cell?	75
	Recognition of cells replicating their DNA	77
	Cell cycle quiescence	84
	Cyclins	86
	Flow cytometry techniques	88
5	The judge, the jury, and the executioner – the	
	genes that control cell death	91
	p53 – The guardian of the genome in embryos and adults	91
	Genes that determine survival or death – the $bcl-2$ family	98
	Apoptotic proteases	103
	The big picture	107
6	Stem cells	115
	What is a stem cell?	115
	Stem cell definition	120
	Stem cells and tissue injury	124
	Self-maintenance probability	126
	A test of functional competence for stem cells: clonogenic	
	cells	126
	Are stem cells intrinsically different from transit cells?	129
	Differentiation options: pluripotency	130
7	An in vivo system to study apoptosis: the small	
	intestine	136
	Proliferative organisation in the gut	136
	Apoptosis in the gut	151

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## CONTENTS

	Apoptosis induced by other cytotoxics: are all cells	
	programmed to die?	162
	Apoptosis in the large bowel and the role of <i>bcl-2</i>	164
	The adenomatous polyposis coli (APC) gene	167
	Small and large intestine cancer incidence figures	169
	Genome protection mechanisms	170
8	Cell death (apoptosis) in diverse systems	184
9	Measuring the levels of cell death (apoptosis)	189
Index		199

## Preface

The death of cells in tissues and organisms was recognised by nineteenth-century histologists and anatomists, particularly those from Germany. On the whole it was regarded as a relatively unimportant process; an entirely passive phenomenon that occurred as a consequence of individual cells sustaining damage or becoming senescent and dying. This view remained unchanged for more than a century until it was realised that the death of cells in a tissue was part of the counterbalance to cell division in determining the overall rate of growth of the tissue. This came to prominence in studies on the growth of tumours, particularly from the work of Gordon Steel, where cell loss was realised, in addition to cell proliferation, to be important in contributing to the overall rate of tumour growth. There are two major elements that counterbalance proliferation. First, there is loss of proliferating cells to a functionally differentiated state with no capacity to return to a proliferative state. Second, there is loss of cells through cell death. Dead cells were recognised in sections of tumours analysed through the microscope in a way that was similar to that described in the early literature. For a time, the concept of cell death as an important factor in growth rate remained the almost exclusive preserve of those working on questions relating to tumour growth. From the early days of microscopic analysis, researchers of the embryological development of organisms from worms and flies to humans, and those studying metamorphosis in the life cycle of organisms such as butterflies and frogs, realised that the loss or the removal of cells was a vitally important phenomenon: this process of cell removal was termed programmed cell death.

#### xii

#### PREFACE

In 1972, a group of pathologists from Aberdeen and Brisbane published a research paper that radically changed the field of research into the processes of cell death. In this paper, they described in detail the changes that occurred in the electron microscopic appearance of individual cells when they died: in this case, the death of kidney tubule cells in response to high levels of a corticosteroid. This and subsequent studies clearly indicated that, far from being a passive phenomenon, cell death could involve considerable cellular activity. The term apoptosis was coined to describe this process of cell death, with active cellular involvement implying a suicide-like process was programmed into cells. There was initial reluctance by the more conservative elements of the scientific community to accept the phenomenon of apoptosis, and for a while programmed cell death, as described by developmental biologists, and apoptosis were thought of as separate processes. Inevitably though, these have now been seen to be essentially the same active process, and since the mid-1980s apoptosis research has flourished into an exponentially expanding field. Surprisingly, it was work in the United Kingdom and Australia that kept the concept of apoptosis alive in the late 1970s and early 1980s. It was only after this that the American scientists entered the field.

It is now recognised that cells may die because they become old and defective, because they are surplus to the requirements of the tissue, or because they incur some damage. Each of these possibilities involves considerable internal cellular programming to regulate gene and protein expression. In addition to internally derived signals, cells can be instructed to commit suicide in response to external signals from their direct neighbours or local cells, from cells of the immune system, and from systemically derived signals.

Apoptosis is an integral part of the regulation of tissue morphogenesis during development and also the regulation of cell production under the stable conditions that one sees in adult organisms in species as diverse as worms, flies, mice, and humans. Abnormalities of tissue growth (e.g., shrinkage or atrophy of tissues with ageing, and diseases of increased proliferation like psoriasis and abnormal CAMBRIDGE

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#### PREFACE

growth like cancer) may all result from an imbalance between the processes of cell division and cell loss by differentiation and apoptosis. Extensive studies are currently underway to determine the molecular and genetic regulation of apoptosis in a variety of situations. Such studies may help in understanding and ultimately preventing the development of tumours and other diseases of proliferation. They may facilitate also the development of entirely new strategies for treating such diseases.

With the evolution and expansion of research into apoptosis, it is becoming an important discipline within the field of cell biology. I have attempted here to describe in simple terms the current status of our knowledge of apoptosis, to explain some background to the field, and to describe some of the difficulties and uncertainties that surround experiments involving apoptosis and the attempts to assess quantitatively the number of cells dying in various circumstances. I have not included extensive lists of references, which clutter and distract from reading the text, but have included various lists of additional reading matter, which generally are either key papers or extensive review articles from which further papers may be identified.

Most of the conclusions that one draws in scientific research represent approximations to the truth. This is particularly true of the field of apoptosis research as it stands at the moment. The search for the truth is, in my view, not particularly facilitated by some of the modern techniques of molecular biology, which commonly are performed on highly specialised cell culture systems and often make use of unconvincing changes in the intensity of blots and gels and make little use of statistical approaches for testing for significance. In apoptosis research, the work can often be based on single or a small number of experiments, the results of which are rarely, if ever, of the 'all or none' type.

It is relatively easy to identify cells that are dying in tissues, but to define the moment at which cell death starts and finishes is extremely difficult. The problems of defining life and death for a cell are similar to the current medical and ethical difficulties associated with defining life and death in humans. Furthermore, the number of cells that one xiii

xiv

#### PREFACE

sees displaying the characteristics of dead cells in a tissue may not be the same thing as the number of cells dying per unit of time, for a variety of complex reasons.

I have been involved in apoptosis research since the mid-1970s and my interests have centred on the role played by apoptosis in a rapidly dividing tissue: the intestinal mucosa. This is probably one of the most extensively studied tissues and provides a nice model biological system for studying cellular interactions. I shall refer to it fairly heavily and because it is a tissue with which I am very familiar it will, inevitably, be something of a personal and idiosyncratic view that is presented.

I have also attempted to explore some of the difficulties outlined above, which on the whole have not been addressed by current researchers. I do this to emphasise the point that nothing is entirely clear and conclusively resolved in science, in the hope that it may stimulate new, young scientists to attempt to address some of the questions and clarify the uncertainties, and so approach closer to the scientific truth. I also hope that some may become interested in understanding the complex biochemical interactions and intercellular dialogues that go on between cells in the body that determine whether they divide, differentiate, or die.

*C*.*S*.*P*.

*Note:* A good background review on apoptosis can be found in Harmon, B. V., and Allan, D. J., Apoptosis: a 20th century scientific revolution. In: *Apoptosis in Normal Development and Cancer*. Taylor & Francis. London, 1–19, 1996.

# Acknowledgements and dedications

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A book like this would not be possible without many years of work in the field, during which I have relied on the hard work of a large number of dedicated and loyal staff and visiting scientists performing the necessary experimental work. These are too many to list but I make an exception for my long-suffering research assistants, xvi

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C.S.P.